ELECTROPHORETICALLY MEDIATED CHEMICAL ANALYSIS

This is a continuation of application Ser. No. 07/944,846 filed on Sept. 14, 1992 now abandoned.

The invention relates in general to techniques for analysis of chemical species, and in particular to analyses involving electrokinetic separation.

BACKGROUND OF THE INVENTION

Capillary electrophoresis is a well-known procedure for separation of chemical components. A sample solution containing molecules to be separated is placed in a length of capillary tubing containing an electrophoretic medium. Upon application of an electric field across the capillary, different components within the sample migrate at distinct rates towards the oppositely charged end of the capillary dependent upon their relative electrophoretic mobilities in the electrophoretic medium. Due to the varying electromigratory rates, the sample components become increasingly separated into distinct zones or groups as they progress along the capillary. At some position along the capillary, the components of the sample are detected.

Electrophoresis has been applied to the separation of charged materials such as proteins, nucleic acids, and cells. These separations depend upon differences in charge density, molecular size, and partitioning or complexation with a mobile phase additive. U.S. Pat. No. 5,061,361 relates to a capillary zone electrophoresis system in which a nanoliter volume of sample is introduced into the capillary tube, and an electric field is imposed on the system to effect separation of the charged components. After migration along the length of the tube, the sample components are detected via ultra-violet absorbance. U.S. Pat. No. 5,084,150 relates to an electrokinetic method of separation in which the surface of moving charged colloidal particles is treated so as to interact selectively with the sample molecules to be separated. An electric field is imposed on a capillary tube containing the colloidal particles and the sample to achieve separation. U.S. Pat. No. 5,045,172 relates to a capillary electrophoresis apparatus in which electrodes are attached at each end of a capillary tube, and a detector is coupled to the tube. U.S. Pat. No. 4,181,589 relates to a method for separating biological cells using an electric field. The above-described U.S. patents are hereby incorporated by reference.

One object of the invention is to perform chemical reactions in a capillary so as to allow detection of a product, while utilizing only a very small volume of sample and a small quantity of analytical reagent. Two components may be mixed, e.g., a sample and a reagent which reacts stoichiometrically with an analyte in the sample, to produce a product, and the product electrophoretically separated and measured so as to indicate the amount of analyte originally present in the sample. Another object of the invention is to perform a chemical analysis for an analyte in a sample without finding it necessary to direct a product based on a unique direction characteristic of the product. Another object is to electrophoretically separate, product from sample and reactant quickly at a high voltage, without subjecting the sample to high temperature. The invention also allows for virtually instantaneous mixing of chemical components without an active process of mixing and with a minimum of dilution and diffusion of sample and/or product. Yet another object of the invention is to regenerate a capillary electrophoretic system without the need for flushing the system. Another object is to move a sample through a capillary electro-osmotically and thus without the need for a mechanical pump. Yet another object is to perform chemical analysis on multiple components of the same sample substantially simultaneously.

SUMMARY OF THE INVENTION

The invention encompasses methods of analysis of an analyte in a sample, and is based on the discovery that analyte determination may be performed rapidly using capillary electrophoresis on exceedingly small amounts of sample by converting analyte in the sample to a product that is detectable.

Methods of the invention relate to a novel concept of chemical analysis in which an analyte and a reactant are brought into contact electrophoretically and a chemical reaction is allowed to occur in which a covalent bond is formed or broken and a product is formed or depleted. The product is transported with a characteristic velocity in an electric field to a detector. This process, which is the basis for the analysis methods of the invention, is termed electrophoretically mediated chemical analysis (EMCA), and involves three stages: a pre-reaction stage in which the zones of analyte and reactant are merged by electrophoretic mixing, a reaction stage in which a covalent bond is formed or broken and a product is produced or depleted, and a post-reaction stage in which the product is electrophoretically transported to a detector and detected. During the pre-reaction interval, it is possible to separate analytes where more than one analyte is present in the sample. In the post-reaction phase, separation of the product from unreacted analyte, reactant, or any other chemical species involved in the reaction may also be effected.

Unlike traditional electrophoresis, electrophoresis utilized according to the invention exploits inherent or induced differences in electrophoretic velocities of product and/or analyte and reactant in a given electrophoretic medium in order to mix and separate these components. The electrophoretic mixing of chemical species confers a special advantage over the traditional methods in the field of chemical analysis; i.e., electrophoretic mixing of analyte and reactant is performed without substantial dilution of the chemical species contained within the zones. Under the influence of an applied potential and a chosen electrophoretic medium, a chemical species may possess a distinct electrophoretic mobility which will allow it to electrophorese essentially independently of the bulk solution. Thus, a zone of analyte and a zone of reactant, which move in the electric field with different electrophoretic mobilities, may become interpenetrated without the volumetric addition of the bulk solutions. In addition, electrophoretic mixing does not require turbulent flow to fully merge two zones. Thus, the inventive methods of chemical analysis are simpler and more efficient than conventional chemical analysis methods. At high potentials, e.g., 300–2000 volts/cm, full electrophoretic mixing of two zones can often be achieved in milliseconds.

The electrophoretic separation capability of methods of the invention allows for separation of chemical species prior to as well as after the chemical reaction. This powerful ability confers many advantages which are absent in previous methods of chemical analysis. Prior to the chemical reaction, chemical species that interfere with the chemical reaction may be electrophoresed away from the analyte of interest, thereby enhancing the selectivity of the reaction. Interfering reaction side-products also may be separated from the detectable product following the chemical reaction. After, the chemical reaction has commenced or been